IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Elliott Bennett-Guerrero et al. Art Unit : 1645
Serial No. : 09/423,546 Examiner : Rodney P. Schwartz
Filed : November 12, 1999
Title : VACCINE AGAINST LIPOPOLYSACCHARIDE CORE

Commissioner for Patents
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DECLARATION OF DR. BENNETT-GUERRERO

1. I am a named inventor for the above-captioned patent application.

2. I am currently an anesthesiologist and critical care physician on the faculty at Duke University Medical Center in Durham, North Carolina, actively involved in research, and I am the Director of Perioperative Clinical Research at the Duke Clinical Research Institute.

Prior to this I was Director of Cardiac Anesthesia at Columbia University College of Physicians & Surgeons in New York. Following graduation from Harvard Medical School, I trained in cardiac anesthesiology and critical care medicine at Duke University Medical Center. I am board certified in both anesthesiology and critical care medicine and I am an active clinician.

My research focuses on the etiology and role of systemic inflammation in the perioperative period, in particular, the roles of intravascular volume status, splanchnic (intestinal) perfusion, endotoxemia, and immunity to endotoxin.

3. I am aware of the following experiments relevant to the invention.

4. The above-captioned patent application as well as a published article -- Bennett-Guerrero et al., *Infection Immunity*, 2000, 68:6202-6208 -- report experiments in which rabbits were immunized with cocktails of complete core LPS. In one experiment, the cocktail included complete core LPS from *E. coli* K12, *E. coli* R1, *P. aeruginosa* PAC 608 and *B. fragilis*. Immunization generated antibodies that

cross-reacted with LPS from other bacteria including other *E. coli* serotypes and other bacterial species. See Figs. 3 and 4 and accompanying text at pp. 6204-6205.

5. Bennett-Guerrero et al. (cited above) also reports experiments in which mice vaccinated with complete core LPS from one strain (*E. coli* K12) or with a cocktail of complete core LPS from *E. coli* K12, *E. coli* R1, *P. aeruginosa* PAC 608 and *B. fragilis*. The mice were challenged with LPS from a different strain of (*E. coli* 018) and the vaccine protected against that challenge.

6. The above findings are relevant to the invention because they show both the cross-reactivity and the protective capacity of the immune response induced by complete core rough LPS, particularly a cocktail of compelete core rough LPS taken from the group: *E. coli*, *P. aeruginosa* and *B. fragilis*.

7. I have read the office actions on the above application, including paper 13 (September 27, 2001), paper 16 (February 25, 2002) and paper 18 (June 18, 2002). I understand that the Examiner complains that the above experiments are limited to the use of LPS incorporated in liposomes. Accordingly, I report the following experiments in which the vaccine comprises LPS complexed to a carier protein such as CRM or BSA. I also understand that the Examiner complains that the above experiments are limited to rough complete core LPS from certain strains of *E. coli*, *P. aeruginosa* and *B. fragilis*, whereas the claims cover cocktails of rough, complete core LPS from *E. coli*, *P. aeruginosa* and *B. fragilis*. Accordingly I report the following additional experiments.

8. **Example 1 - Preparation and immunogenicity of complete core LPS conjugates using different carrier proteins**

K12 complete core LPS was detoxified and complexed to protein by standard techniques. Groups of mice were immunized with the complexed LPS and their sera was collected. As shown in Appendix A, animals immunized with complete core LPS antigen elicited antibodies to LPS from several unrelated gram-negative bacterium. Formulations that were administered without the adjuvant alum (Lanes C and D), were particularly immunogenic compared with those containing alum (Lanes E and F). In addition, formulations using CRM (Lanes D and F)

were particularly immunogenic compared with those using BSA (Lanes C and E), therefore CRM was used for subsequent experiments.

9. **Example 2 - Preparation of K12 and R1 complete core LPS conjugates and immunogencity of a cocktail of complete core LPS conjugates**

E. coli K12 and *E. coli* R1 complete core LPS's were detoxified and complexed to protein by standard techniques. Rabbits were immunized with the resulting cocktail of complexes of complete core LPS complexes from *E. coli* K12 and R1. Sera for each group were pooled and tested for the presence of antibodies to several clinically relevant LPS's.

As shown in the figure in Appendix B, animals immunized with that cocktail of complete core LPS complexes [Ra LPSs (C)] elicited antibodies to LPS from several unrelated gram-negative bacterium, including *E. coli, Pseudomonas aeruginosa, Klebsiella pneumoniae*, and *Bacteroides*. The figure also shows reactions that were generally similar to reactions to immunization with a cocktail of complete-core LPSs incorporated into liposomes [Ra LPSs (L)] as described in Bennett-Guerrero et al. *Infection Immunity*, 2000, 68:6202-6208.

10. **Example 3 - Protection from Lethal Dose of Unrelated LPS After Vaccination with K12 and R1 Cocktail of Complete Core LPS Conjugates**

The ability of a complete core (Ra chemotype) conjugate vaccine formulation to protect mice from a lethal dose of LPS from an unrelated gram-negative bacterial species was tested using the established mouse galactosamine model

The 100% lethal dose of LPS in mice was determined by standard techniques. Mice were immunized with a vaccine comprising complete core LPS from K12 and R1 LPS detoxified and conjugated by standard techniques. After immunization, the mice were administered galactosamine and *Klebsiella pneumoniae* serotype O1 LPS.

As shown in the figure in Appendix C, the conjugate vaccine, comprising a cocktail of complete core LPS from *E. coli* strains K12 and R1 protected mice from a lethal dose of LPS from an entirely different species of bacteria (*Klebsiella pneumoniae* serotype O1). This result is consistent with data shown above demonstrating that a conjugate vaccine, comprising a cocktail of complete core

LPS from *E. coli* strains elicited antibodies to LPS from this common isolate of *Klebsiella pneumoniae* serotype O1, as well as to common gram bacterial isolates including *E. coli, Pseudomonas aeruginosa*, and *Bacteroides*.

11. **Example 4 - Protection from Lethal Dose of Unrelated LPS After Vaccination with *E. coli* K12 and *Pseudomonas aeruginosa* Cocktail of Complete Core LPS Conjugates**

The ability of a complete core conjugate vaccine formulation to protect mice from a lethal dose of LPS from an unrelated gram-negative bacterial species was tested using the established mouse galactosamine model as described in Example 3, above, using a cocktail of *E. coli* K12 and *Pseudomonas aeruginosa* LPS complexes. As shown in the figure in Appendix D, this conjugate vaccine, comprising a cocktail of complete core LPS's from *E. coli* and *Pseudomonas aeruginosa* protected mice from a lethal dose of LPS from an unrelated bacteria (*E. coli* serotype O18). This result is consistent with data demonstrating that a conjugate vaccine, comprising a cocktail of these complete core (Ra) LPSs elicited antibodies to LPS from this common isolate of *E. coli* serotype O18, as well as to other common gram bacterial isolates.

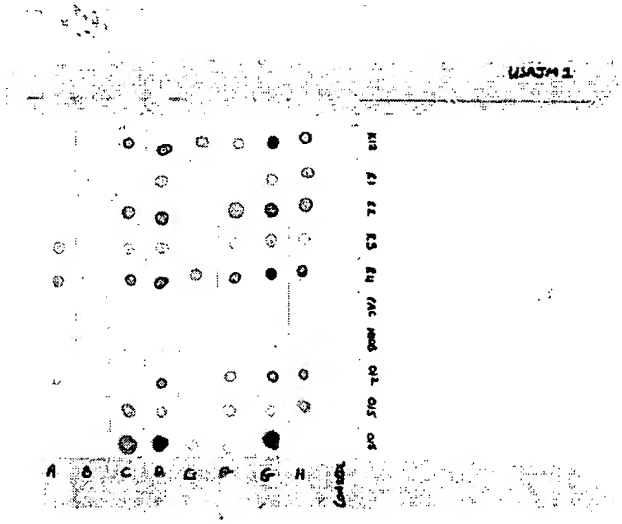
12. The above experiments show that cocktails of rough complete core LPS complexes are effective in generating an immune response that is protective and cross-reactive among species and serotypes. Those skilled in the art would understand that the invention is not limited to LPS presented in liposomes.

13. The above experiments also show that the invention is not limited to any single bacterial species or serotype or any specific cocktail.

Elliott Bennett-Guerrero, M.D.

APPENDIX A: Example 1

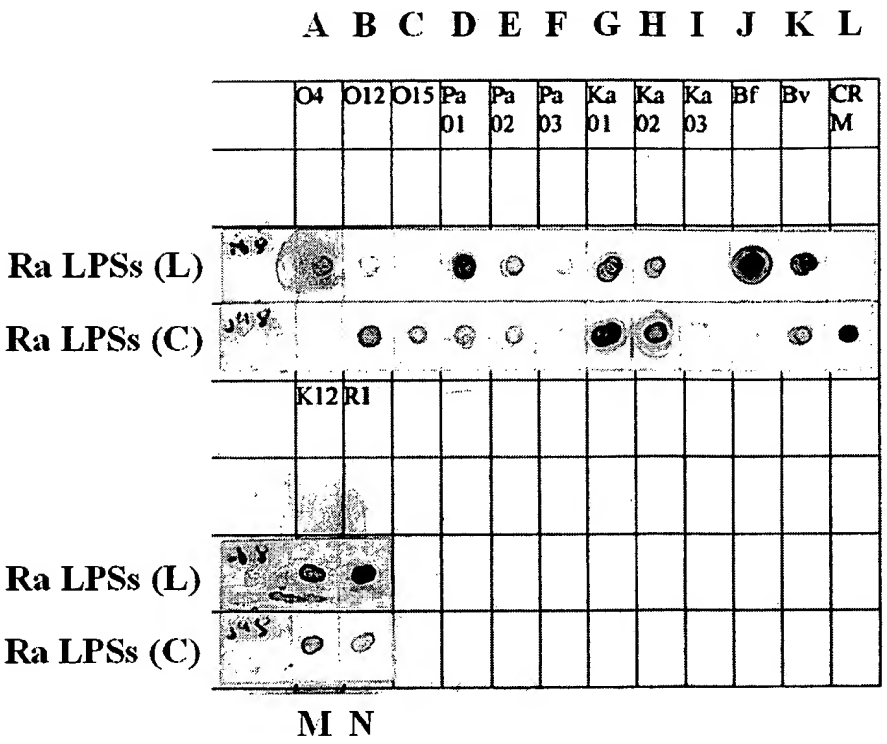
IgG



A BSA
B CCM
C K12/BSA
D K12/CCM
E K12/BSA
F K12/CCM
G K12 HEAT KILLED CELLS
H R1 HEAT KILLED CELLS

MOUSE SERUM POOLED USED AT $\frac{1}{50}$ - O/N; RT
α-MOUSE IgM $\frac{1}{2000}$ - 3h; RT

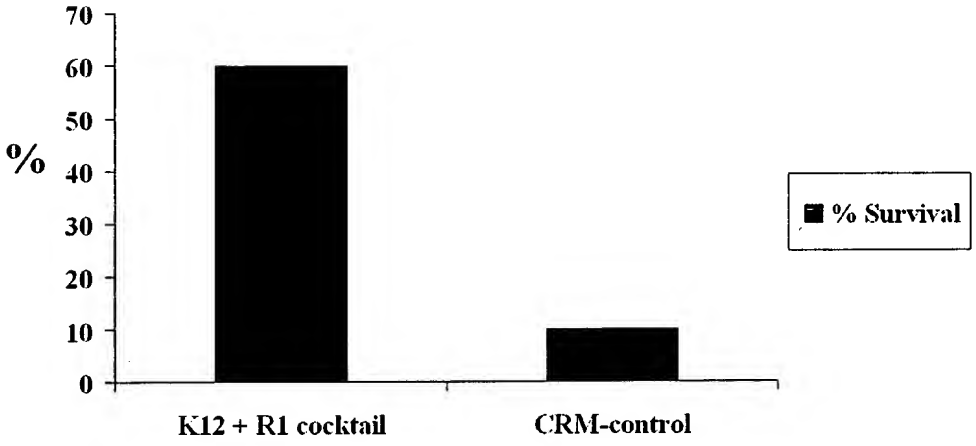
APPENDIX B: Example 2



Lane A: *E. coli* O4 LPS
Lane B: *E. coli* O12 LPS
Lane C: *E. coli* O15LPS
Lane D: *Pseudomonas aeruginosa* O1 LPS
Lane E: *Pseudomonas aeruginosa* O2 LPS
Lane F: *Pseudomonas aeruginosa* O3 LPS
Lane G: *Klebsiella pneumoniae* O1 LPS
Lane H: *Klebsiella pneumoniae* O2 LPS
Lane I: *Klebsiella pneumoniae* O3 LPS
Lane J: *Bacteroides fragilis* LPS
Lane K: *Bacteroides vulgatus* LPS
Lane L: CRM control
Lane M: *E. coli* K12 LPS
Lane N: *E. coli* R1 LPS

APPENDIX C: Example 3

K12 and R1 Complete Core (Ra) LPS Conjugate Vaccine Protects Mice From Lethal Challenge With 2xLD100 *Klebsiella pneumoniae* serotype O1 LPS



APPENDIX D: Example 4

K12 and PAC608 Complete Core (Ra) LPS Conjugate Vaccine Protects Mice From Lethal Challenge With 2xLD100 *E. coli* O18 LPS